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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:

Candice A.C. Gardner Gary Edward Henke

Date:

March 14, 2003

Serial No.:

09/758.804

Group Art Unit:

1638

Filed:

January 11, 2001

Examiner:

Ashwin D. Mehta

For:

"INBRED MAIZE LINE PH6JM"

Assistant Commissioner for Patents Washington, D.C. 20231

RULE 132 DECLARATION OF DR. DINAKAR BHATTRAMAKKI

Sir:

- I, Dinakar Bhattramakki, Ph.D., do hereby declare and say as follows:
- 1. I am skilled in the art of the field of the invention. I have a Ph.D. in Plant Molecular Genetics from the University of Illinois at Urbana-Champaign. I have a Bachelor of Science degree in Agricultural Sciences from the University of Agricultural Sciences, Bangalore, India. Since 1997 I have been engaged in the analysis of molecular markers for plants. I have supervised the Molecular Marker Applications lab at Pioneer Hi-Bred International, Inc. from January 2002 until the present.
- 2. I am familiar with the methods used in the analysis of Simple Sequence Repeat, SSR, marker data for inbred PH6JM conducted at Pioneer Hi-Bred International, Inc. The analysis of the SSR profile of inbred PH6JM may be accomplished without any undue experimentation. The SSR profile for inbred PH6JM is attached hereto.

PCR $^{\prime\prime}$ detection of SSRs is accomplished by using two oligonucleotide primers

Appendix B



flanking the polymorphic segment of DNA. Amplification is accomplished through repeated cycles of heat denaturation of the DNA followed by annealing of the primers to their complementary sequences at low temperatures, and extension of the annealed primers with DNA polymerase.

- 4. Markers are scored following amplification and gel electrophoresis of the amplification products. Scoring of marker genotype is based on the size or weight of the amplified fragment. While variation in the primer used or in laboratory procedures can affect the reported marker score, relative values remain constant regardless of the specific primer or laboratory used.
- 5. Primers that may be used to identify the SSR markers reported herein are publicly available and may be found in the Maize DB on the World Wide Web at agron.missouri.edu/maps.html (sponsored by the University of Missouri), in Sharopova et al. (Plant Mol. Biol. 48(5-6):463-481) and/or in Lee et al (Plant Mol. Biol. 48(5-6); 453-461). Markers shown for PH6JM are the publicly available markers in the sources listed above for which PH6JM was tested and shown to be homozygous.
- Map information is provided by bin number as reported in the Maize DB. The bin number digits to the left of decimal point typically represent the chromosome on which such marker is located, and the digits to the right of the decimal typically represent the location on such chromosome.
- 7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date:	Ву:	BAR
		Dinakar Bhattramakki



Public Name of	bin#	PH6JM base
Marker		pairs
phi427913	1.01	129.38
bnlg1014	1.01	114.07
phi056	1.01	246.94
bnlg1083	1.02	221.86
bnlg1127	1.02	114.36
bnlg1429	1.02	204.64
bnlg1627	1.02	202.70
phi109275	1.03	126.31
phi339017	1.03	
bnlg1203	1.03	308.35
bnlg1484	1.03	
bnlg1832	1.05	
bnlg1886	1.05	
bnlg1041	1.06	
bnlg1615	1.06	
bnlg1556	1.07	
phi323065	1.08	
phi335539	1.08	
phi423298	1.08	
phi002	1.08	
bnlg1331	1.09	
phi011	1.09	
phi308707	1.10	
phi227562	1.11	
phi064	1.11	
phi402893	2.00	
phi96100	2.01	+
bnlg1017	2.02	
bnlg2277	2.02	
bnlg1064	2.03	
bnlg1018	2.04	-
bnlg1909	2.05	
bnlg1138	2.06	+
bnlg1396	2.06	
bnlg1831	2.06	
phi251315	2.07	
phi328189	2.08	-
phi427434	2.08	
phi435417	2.08	<u> </u>
bnlg1141	2.08	
bnlg1940	2.08	
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phi453121	200	
phi104127	3.01	<u> </u>
phi404206	3.01	303.14



Public Name of	bin #	PH6JM base
Marker]	pairs
phi193225	3.02	136.71
phi374118	3.02	213.36
bnlg1144	3.02	134.68
bnig1647	3.02	137.52
bnig1523	3.03	264.89
bnlg1019	3.04	179.98
bnlg1035	3.05	101.06
phi053	3.05	171.68
phi102228	3.06	126.84
bnlg1160	3.06	222.23
bnlg1951	3.06	121.12
bnlg2241	3.06	142.63
phi072	4.00	139.43
phi213984	4.01	284.60
phi295450	4.01	184.83
bnlg1162	4.03	92.86
phi308090	4.04	218.64
phi096	4.04	234.58
phi438301	4.05	211.81
bnlg1159	4.05	147.81
bnlg1 7 55	4.05	240.07
bnlg1937	4.05	227.36
bnlg1265	4.05	223.71
phi079	4.05	177.74
bnlg1189	4.07	131.56
bnlg2244	4.08	199.58
bnlg1006	5.00	229.38
phi109188	5.03	161.52
phi331888	5.04	130.72
bnlg1208	5.04	118.88
phi333597	5.05	210.74
phi085	5.06	250.16
bnlg1118	5.07	82.56
bn/g1711	5.07	176.65
phi423796	6.01	128.39
phi389203	6.03	306.15
phi452693	6.04	130.98
phi445613	6.05	96.95
bnlg1174	6.05	218.72
phi299852	6.07	117.30
phi364545	6.07	131.46
bnlg1740	6.07	230.27
bn/g1759	5.07	125.81
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phi328175	7.04	128.31
phi260485	7 05	285.39

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Public Name of	bin #	PH6JM base
Marker		pairs
phi051	7.05	138.57
phi069	7.05	195.31
phi116	7.06	164.92
phi420701	8.00	289.29
bnlg1194	8.02	174.99
phi100175	8.03	145 34
bnlg2082	8.03	172 52
phi115	8.03	290.66
phi121	8.03	93.90
bnlg2046	8.04	306.22
bnlg1176	8.05	217.94
bnlg1152	8.06	148.42
bnlg1065	8.07	226.87
bnlg1056	8.08	94.48
phi015	8.08	100.05
phi233376	8.09	136.10
bnlg2122	9.01	203.41
bnlg1012	9.04	161.54
phi032	9.04	231.00
phi108411	9.05	126.04
phi236654	9.05	117.08
bnlg1375	9.07	164.93
bnlg1129	9.08	300.56
phi041	10.00	209.99
phi96342	10.02	249.64
phi059	10.02	143.60
bnlg1079	10.03	170.34
bnlg1655	10.03	126 51
phi050	10.03	83.27
phi301654	10.04	128 33
phi062	10.04	157.81
phi323152	10.05	134.72
bnlg1185	10.07	188.95
phi109642	2.03/2.04	148.23
bnlg1720	1.09/1.10	236.44
phi448880	9.06/9.07	176.99